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Estimation of Plasma Half-Lives of Vasopressin Analogs from Vasopressor Responses by Curve-Fitting (40102)

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Finding plasma half-lives by measuring peptide concentrations in plasma after injection or infusion has serious limitations when one wishes to study rapidly-eliminated peptides, such as vasopressin, in a small animal. The time needed to draw blood samples becomes an important source of imprecision. The volume of blood which one must take is also a limiting factor. One usually cannot repeat half-life measurements on one animal or compare two peptides in one animal, because this would require taking too much blood. If one could estimate plasma half-lives from a biological response one could avoid these limitations of direct plasma measurements.

In this paper we describe a method for estimating plasma half-lives by comparing the rate of decay of a biological response to the dose-response curve for the same peptide in the same rat. This method has been applied to arginine-vasopressin and two of its analogs using vasopressor responses in rats.

Materials and methods. Female rats weighing about 200 g were anesthetized with sc urethane (175 mg/100 g) and the trachea, carotid artery, and jugular vein were cannulated. After surgery, 1 ml of a 1 mg/ml solution of phenoxybenzamine (Dibenzyline, Smith Kline and French) was given sc. The carotid cannula was connected to a Statham pressure transducer and blood pressure was recorded on a Grass polygraph.

In the first experiment, which included nine rats, two rats were usually studied simultaneously. After their basal blood pressures became stable, one rat received arginine–vasopressin (AVP), the other, 1-deamino-arginine vasopressin (dAVP). Both peptides were diluted in 0.05 *M* acetic acid, 0.5% chlorbutanol solution and given in a series of 6 iv injections (2.5, 5, 10, 20, 40, 80 ng sequentially in order of ascending dose) at 15-min intervals. Injections were given rapidly. The total volume of injection plus wash did not exceed 140 μl. This volume of chlorbutanol solution given alone did not alter blood pressure. One hour was allowed to elapse after the last injection of the series. During this time, blood pressure returned to basal levels. The injections were then repeated with each rat receiving the other peptide in a series of injections identical to the previous series.

From the resulting blood pressure tracings, of which Fig. 1 is an example, tables of mean blood pressure vs. time were made for each dose for each rat. Mean blood pressure was measured at 2-min intervals beginning at the time of maximum response. The baseline blood pressure was taken to be the mean blood pressure immediately preceding the injection. Mean blood pressure was calculated using the formula: mean blood pressure = diastolic blood pressure + ⅓ (systolic blood pressure − diastolic blood pressure).

From the tabulated blood pressure data, log dose vs peak response graphs (as in Fig. 2) and response vs time graphs (as in Fig. 3) were made for each drug in each rat. All subsequent calculations were made with data from these tables or graphs. In a few rats, the response to dAVP did not decline in 15 min. The data from these rats were discarded.

The second experiment was similar to the first except that the rats were injected with arginine-vasotocin (AVT). Seven rats were each given two series of injections. The first series of six doses (3.75, 7.5, 15, 30, 60, 120 ng) was sequential as in Experiment 1. In the second series the six doses were given in a random order determined by assigning each dose a number generated by a random number generator and reordering the doses according to the assigned numbers. In this experiment, the rats were allowed to rest 15 min at basal blood pressure levels between successive injections.

AVP (376 vasopressor U/mg), dAVP (330

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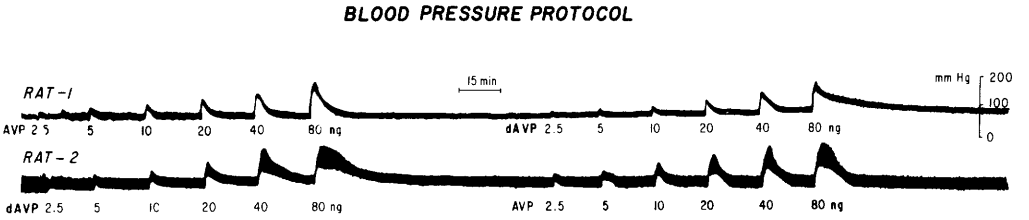

FIG. 1. Blood pressure tracings, Rats 1 and 2. Rats were pretreated with phenoxybenzamine and given a series of injections of AVP and dAVP.

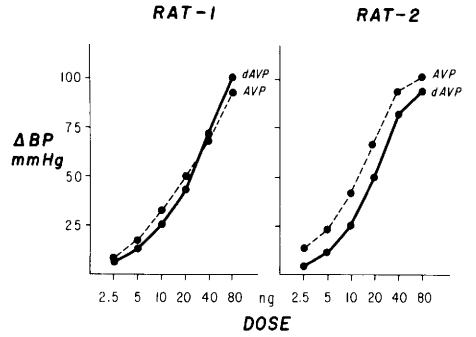

FIG. 2. Log Dose vs Response. The ordinate records the maximum blood pressure increments which occurred in response to a series of injections of 2.5, 5, 10, 20, 40, and 80 ng of AVP or dAVP.

U/mg), and AVT (227 U/mg) were synthesized by Maurice Manning and co-workers (1, 2).

AVP and dAVP were used initially to establish this method for estimating half-lives from responses because their half-lives had been previously estimated from direct plasma measurements from the initial part of the curve of disappearance from plasma following cessation of prolonged constant infusions of the peptides and had been found to differ significantly (3).

Results. Inspection of the blood pressure tracings from the first experiment showed that the higher doses of dAVP produced longer vasopressor responses than did doses of AVP which were equipotent in terms of maximum responses in every rat (as, for example, in Fig. 1). The longer responses to dAVP were consistent with its longer half-life previously estimated from infusion experiments (3). We then attempted to demonstrate that vasopressor responses could be analyzed to yield independent estimates of plasma half-lives. To do this we considered that the vasopressor response could be divided into three phases: (1) a period during which pressure reaches a peak and the iv injection mixes in the plasma volume, (2) a period in which peptide levels fall toward threshold and the pressure declines, and (3) the time it takes for the vasopressor response to disappear completely after the plasma concentration has fallen below threshold levels. In a series of injections of increasing doses, we assume that phase 1 and phase 3 remain nearly constant in duration, but phase 2 lengthens. By comparing the time courses of responses to a series of injections, the influence of the drug half-life (which determines the length of phase 2) can thus be separated from the other components of the response.

In order to find a numerical value for each half-life, we made two other assumptions. The first was that, in a given rat, equal elevations of blood pressure reflected equal plasma peptide concentrations, whether the elevation was the maximum response to an injection or occurred during the declining phase of the response to a larger injection. Development of peak pressure or the rate of decline may both lag behind the peak and decline phases of plasma peptide concentration. If these lag times remain constant they would not affect our estimates of half-life. The second was that the logarithm of the plasma peptide concentration fell linearly with time after the maximum response was reached. This would occur if the three routes of exit from the plasma, metabolic clearance, urinary clearance, and diffusion into the extravascular space, were all exponential processes.

To estimate half-life, a log dose-response curve (Fig. 2) was constructed from the maximum responses to a series of iv injections. If log plasma concentration falls linearly with time after iv injection the time course of the decay of the response to the largest injection of the series used to construct the log dose-

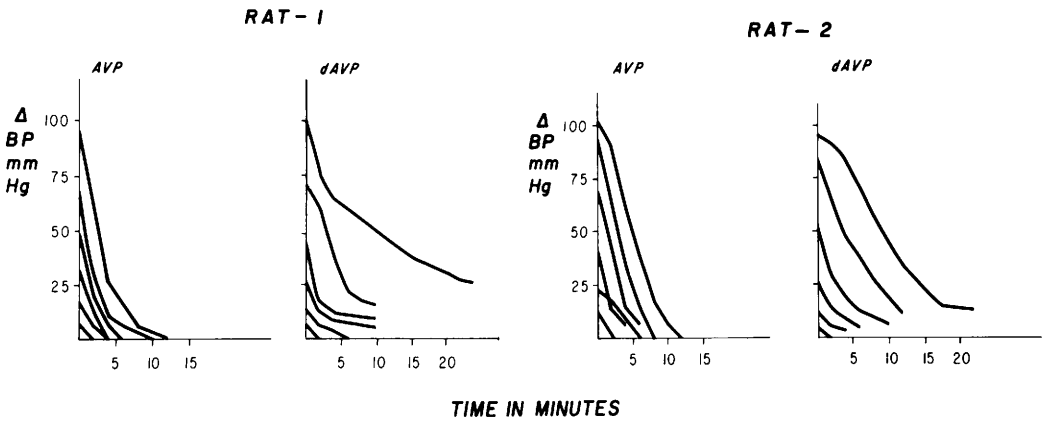

FIG. 3. Response vs Time. Each graph shows the time course of responses to a series of injections (2.5, 5, 10, 20, 40, and 80 ng) of AVP or of dAVP. The time at which the response reached a maximum is taken as time 0.

response curve is a continuous "unrolling" of the log dose-response curve. The two curves should be superimposable if a proper fixed interval between doublings of dose in the log dose-response curve is chosen.

Curve 1 (in Fig. 4) shows the decline in response as log dose (which is proportional to log plasma concentration) declines. If log plasma concentration fell linearly with time, the same response curve would be generated, but time would be the abscissa. Each successive point on Curve 1 represents the response to half the dose of the previous point. Halving of the plasma concentration is what would occur in one half-life. Curve 2 is the decay of the response after peptide injection. If the correct half-life is chosen, the points of Curve 1 can be fitted at half-life intervals on Curve 2. This curve-fit method involves fitting the middle four responses of the log dose-response curve (that is, the maximum responses to 5, 10, 20, and 40 ng, in the case of AVP and dAVP) on the response vs. time curve by postulating a fixed time interval between them (Fig. 5). The time interval which results in the best fit is taken to be the half-life. The fitting was done by trial and error. Usually we started by fitting responses to doses 3 and 4 exactly on the response vs time curve and used the time interval between the fitted points as our initial guess for the half-life. The interval was then adjusted as needed to bring responses to doses 2 and 5 into closer agreement with the time curve.

The decay of the response curves could be fitted quite closely in the middle range of the

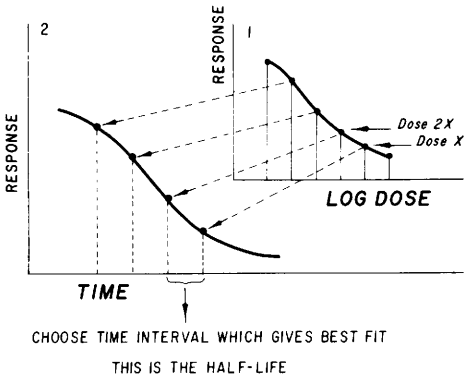

FIG. 4. Curve-fit Method. The middle four points of the log dose-response curve are fitted on the response vs. time curve by postulating a fixed time interval between the points. The time interval which results in the best fit is taken to be the half-life.

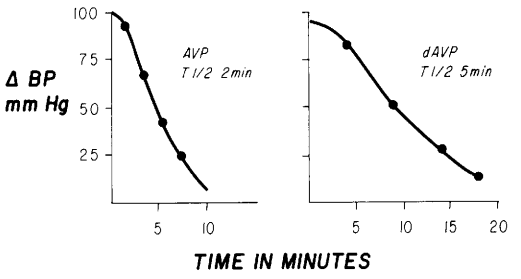

FIG. 5. The curve fit method applied to Rat 2. The solid lines represent the decay of the vasopressor response to 80 ng of each peptide. The filled circles are peak responses to single injections in the same rat. These are plotted at the time intervals that approximate the best fit to the decay curve.

log-dose response curves. This suggests that our assumptions that blood pressures during the decline do reflect plasma peptide levels and that these do decline exponentially in this range are at least approximately true. After prolonged infusions the measured plasma concentrations of AVP and dAVP (3) do not decay at a single rate, but decay more rapidly initially and then more slowly. From these rates "early" and "late" half-lives can be obtained. In the present single injection experiments the rates of decay appear to be nearly exponential and were about twice the rates corresponding to the "early" half-lives estimated from infusion experiments.

Half-lives obtained by the curve-fit method for individual rats are shown in Table I. Half-lives estimated thus for AVP, dAVP, and AVT are 2.6 ± 0.3 (SEM), 9.2 ± 1.4, and 4.0 ± 0.3 min.

Discussion. The 2.6-min half-life for AVP estimated from the vasopressor response falls within the range of previously published estimates based on measurement of plasma concentrations following single injections in normally hydrated rats. These vary from 1.0 to 3.7 min (4). The variation among estimates of plasma half-lives following iv injections presumably reflects differences in anesthesia, presence or absence of adrenergic blockade, the intervals at which plasma samples were obtained, and the limitations of the several assay methods. Data on the post-injection half-life of dAVP in rats are not available.

TABLE I. EFFECTS OF THE ORDER IN WHICH PEPTIDES WERE ADMINISTERED ON HALF-LIVES (IN MINUTES) ESTIMATED BY THE CURVE-FIT METHOD.

A. Order in which AVP and dAVP were injected.

AVP given 1st			dAVP given 1st		
Rat	AVP	dAVP	Rat	AVP	dAVP
1	1.5	12.0	2	2.0	5.0
7	2.0	12.0	3	2.5	6.0
9	3.5	6.0	8	2.0	8.0
11	3.5	6.0	13	4.0	18.0
12	2.5	10.0			
Mean	2.6	9.2		2.6	9.2
±SEM	±0.4	±1.4		±0.5	±3.0

B. AVT administered in ascending and random orders of doses.

Rat	Ascending	Random
14	4.0	3.0
15	4.0	3.0
16	3.5	4.0
17	4.0	4.0
18	3.0	3.0
19	5.0	5.0
20	5.5	5.0
Mean ± SEM	4.1 ± 0.3	3.9 ± 0.3

The half-lives obtained from vasopressor responses, 2.6 min for AVP and 9 min for dAVP, are shorter than, although proportional to, "early" half-lives measured directly from plasma concentrations after constant iv infusions, which are 4.5 min for AVP and 18.4 min for dAVP (3). The reason that postinfusion half-lives are longer may be that peptide accumulates in the extravascular space during infusions and diffuses back into the plasma after the end of the infusion. This would produce a longer half-life as estimated from the rate of decay of plasma peptide concentrations. When single injections are given, however, the early fall in plasma concentrations would reflect metabolic and excretory clearances and diffusion of peptide into the extravascular compartment. This would shorten plasma half-life. Thus neither infusion nor injection experiments necessarily yield a physiologically relevant value for the half-life of a peptide, one may overestimate, the other may underestimate. The plasma half-life of endogenous AVP would appear to depend, therefore, on whether it is released continuously, as if infused, or in spurts, as if injected iv.

In the first experiment, the order in which the peptides were given did not introduce a bias into the results (Table IA). In the second experiment, sequential dosing (low dose to high) did not yield half-lives which were significantly different from those obtained from responses to doses given in random order (Table 1B). When the half-life measurements for AVT were repeated on single rats, the average standard deviation was ±0.3 min. This is less than the standard deviation for measurements between rats (±0.9 min). This was calculated for the seven rats given AVT using a half-life for each obtained by averaging the "ascending" and "random order" half-life values. For comparison, using bioassay of plasma samples, Forsling et al. (5) found a half-life for AVP of 1.6 ± 0.8 (SD) min.

When using the curve-fit method, one must take into account the fact that repeated injections of high doses of peptide at close inter-

vals can cause tachyphylaxis of the response. Before one obtains half-lives for any peptide by the curve-fit method, one must first find a dosing interval long enough to prevent tachyphylaxis. The interval varies with the peptide and must be found empirically.

An advantage of the curve-fit method is that it can separate components of the response. For example, if the plasma peptide concentration included a substantial contribution of peptide from the extravascular space at later times in the course of the response, concentrations would fall with a different "half-life" at later times. This would show up in the curve-fit method as a deviation of the later part of the decay of the response from the curve predicted from the log dose-curve. Also the curve-fit method separates the contribution of the slope of the log dose-response curve to the length of the response from that of the plasma half-life.

The log dose-response relationship influences the length of action in the following manner. If two drugs are similarly distributed and are eliminated at the same rate (that is, the fractions of total drug eliminated per unit of time are equal for the two drugs) and in the same manner, the drug with the less steep regression of response on log will be longer-acting, if one starts from the same degree of biological response for each drug. Since the concentration of each drug in the vicinity of the receptor would fall at the same rate, that drug which acts over a wider range of concentrations (that has a less steep log dose-response relationship) would have a more persistent action. Thus a long drug half-life is not the only factor which may produce a long response, and it is important to be able to separate the effect of drug half-life from the effect of other factors on the length of response.

Other investigators, such as Pliška (6), have measured the half-lives of responses in studies on vasopressor, antidiuretic and uterotonic responses to neurohypophysial peptides. Such measurements are easily made but cannot be readily interpreted in terms of plasma half-lives. Measurements of half-lives of responses would only yield reliable information on relative rates of elimination if all responses were of the same magnitude. Half-lives of responses clearly vary with the magnitude of the initial response (Fig. 3). Comparing half-lives of responses to different peptides would only relate to plasma half-lives if all the peptides had identical log dose-response relationships in all rats. The curve-fit method, although requiring certain unproven assumptions, would appear to provide more meaningful data on the rates of disappearance of peptides from the vicinity of the receptors, and, we presume, from the plasma, since this method factors the decay of the response by the log dose-response relation for each peptide in each rat. Thus variations in log dose-response relations among peptides and among rats do not influence the estimation of half-lives by curve-fitting.

We believe that half-lives estimated by curve-fitting better reflect actual plasma half-lives than do those estimated from the decay of responses alone. In the experiment illustrated in Fig. 5, for example, half-lives of responses to the highest doses of AVP and dAVP were about 5 and 9 min, respectively. These are about twice the half-lives estimated by curve-fitting. The shorter estimate of AVP half-life obtained by curve-fitting is in much better agreement with reported estimates based on actual measurements of plasma AVP levels following single injections (4).

To test directly the assumptions that we have made regarding the relation between vasopressor responses and plasma peptide concentrations would require simultaneous measurements of plasma peptides and vasopressor responses in individual rats. At present this would be technically difficult if not impossible. Nonetheless, we feel that curve-fitting offers substantial advantages over measurement of half-lives of responses alone as a simple and rapid means for estimating plasma half-lives of neurohypophysial hormones and their analogs. This method may also be applicable to studies on half-lives of other rapidly eliminated hormones and drugs.

Summary. Vasopressor responses by rats to graded doses of AVP, dAVP, and AVT were analyzed in an attempt to find a method for estimating plasma half-life from responses. The resulting "curve-fit" method indicated half-lives of 2.6 ± 0.3, 4.0 ± 0.3, and 9.2 ± 1.4 min for AVP, AVT, and dAVP, respectively, after single intravenous injections. The half-lives of two peptides can be compared in

one rat the "curve-fit" method. This method can also provide useful information concerning the relative contributions of the plasma half-life, the log dose-response relationship, and other factors, to the duration of the response.

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